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Phytochemical, Proximate, Mineral Composition and Antioxidant Property of *Moringa Oleifera* Leaf Meal from Two Different Locations in Ghana



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Article History

Received: 7 December 2024

Revised: 29 January 2025

Accepted: 6 March 2025

Published: 12 March 2025

How to Cite

William Kwajo Jimah Kwenin (2025). Phytochemical, Proximate, Mineral Composition and Antioxidant Property of *Moringa Oleifera* Leaf Meal from Two Different Locations in Ghana. *Sumerianz Journal of Medical and Healthcare*, Vol. 8, No. 1, pp. 6-17.

Abstract

Moringa oleifera leaf meal (MOLM) was analyzed in triplicate to determine proximate, mineral, energy, phytochemical profile and antioxidant potential. Data was analysed using STATISTIX 9.0. and means separated by least significant difference at 5 %. Dry matter (9.50 - 9.77 %), crude protein (27.42 - 27.72 %), crude fat (4.55 - 4.65 %), crude fibre (10.37 - 10.56 %), ash (12.98 - 13.39 %), carbohydrate (34.21 - 34.74 %) and gross energy (17.77 - 17.82 MJ/kg) were recorded. Minerals determined were Ca (655.93 - 656.31 mg/kg), Fe (70.21 - 71.60 mg/kg), K (257.72 - 266.58 mg/kg), Mg (69.74 - 73.49 mg/kg), Mn (12.32 - 12.64 mg/kg), P (586.31 - 597.69 mg/kg) and Zn (16.68 - 16.75 mg/kg). Alkaloids, coumarins, flavonoids, saponins, steroids, sterols, tannins and terpenoids were detected. Quantitative analysis gave alkaloids (2.60 - 2.70 %), flavonoids (27.01 - 28.00 mgCE/mg), saponins (1.54 - 1.57 %), tannins (49.26 - 49.76 mgTAE/g), total phenols (106.42 - 106.44 mg/g), oxalate (1.10 - 1.42 mg/g) and phytates (11.51 - 11.72 mg/g). Higher scavenging ability of MOLM of 2,2-diphenyl-1-picrylhydrazyl than vitamin C make MOLM a suitable natural antioxidant. MOLM has adequate amounts of crude protein, minerals and energy, useful phytochemicals and high antioxidant potential.

Keywords: *Moringa oleifera* leaf meal; Proximate composition; Phytochemical profile; Antioxidant assay.

1. Introduction

Moringa oleifera is the most common and widely cultivated of the thirteen species of moringa. It has gained much attention due to its fast growth rate (Bopape-Mabapa *et al.*, 2020). The plant produces leaves all year round including dry season and drought, making it a reliable source of green vegetables. Its ability to survive on marginal lands under adverse weather conditions (Mabapa *et al.*, 2017) large biomass production of leaves and palatability make it important in communities where plant protein is expensive (Su and Chen, 2020). Chemical composition, nutrient profile and low anti-nutritional factors (Peñalver *et al.*, 2022) make moringa leaves a suitable leafy

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vegetable for humans (Kwenin *et al.*, 2011; Sultana, 2020) and as fodder for livestock and other farm animals such as poultry (Paguia *et al.*, 2014; Su and Chen, 2020; Abdoun *et al.*, 2022; Poku *et al.*, 2021; Poku *et al.*, 2023).

The nutritional and pharmaceutical properties of moringa leaves make it a useful resource in animal nutrition. As in other shrub and tree leaf meals such as *Azadiracta indica* (Bonsu *et al.*, 2012) and *Chromolaena odorata* (Bonsu *et al.*, 2013), *Moringa oleifera* leaf meal provides protein, vitamins and minerals (Safa and Tazi, 2014; Sultana, 2020). In addition to containing fibre, moringa leaves contain very useful phytochemicals (coumarin, alkaloids, glycosides, flavonoids, saponins, steroids, sterols and tannins (Gopalakrishnanb, 2016), essential fatty acids, carotene, energy, ten essential vitamins and essential minerals especially Ca, Cu, Fe, K, Mg, P, and S (Gopalakrishnanb, 2016; Peñalver *et al.*, 2022).

Although MOLM has been used in feeding trials with livestock and poultry (especially chicken broilers and layers), proximate composition, nutrient and mineral profiles of MOLM may vary depending on season, edaphic factors, age of leaves, mode of harvesting, drying and processing into leaf meal. This study was carried out to determine the proximate and mineral composition, phytochemical profiles and antioxidant potential of MOLM from two different locations in Ghana before their usage in feeding trials with chicken broilers and layers.

2. Materials and Methods

MOLM was obtained from two sources: locally processed moringa leaf meal at the College Farm of the Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development (AAMUSTED Mampong Campus in the Ashanti Region) as MOLM1 and from NAYEBA Moringa Powder Ltd (commercial producer and exporter of Moringa Powder in Dormaa Ahenkro in the Bono Region of Ghana) as MOLM2. For the production of both MOLM1 and MOLM2, harvested leaves were separated from branches and twigs to reduce chaff, shade-dried for 5 days on concrete floors and regularly turned to prevent them from getting mouldy. Dried and crisp leaves were hammer-milled and sieved through 2.0 – 3.0 mm diameter mesh to constitute *Moringa oleifera* leaf meal (MOLM) and stored in air-tight bags to avoid any possible contamination from foreign materials. Proximate analysis, phytochemical, energy, mineral and antioxidant properties of the two moringa leaf meals were determined before mixing in equal quantities (weight-by-weight) to form a composite leaf meal for inclusion in poultry diets.

2.1. Proximate Analyses

2.1.1. Moisture

Moisture content was determined using official methods of Association of Official Analytical Chemists (1990). Triplicate 10 g MOLM samples were oven-dried (Wagtech®, UK) at 105°C till constant weight. The percentage of moisture content was calculated as:

$$\text{Percent Moisture} = \frac{(\text{Weight of sample} - \text{Weight of dried sample})}{\text{Weight of sample}} \times 100$$

2.2. Crude Fat Content

Two grams (2.0 g) of MOLM samples were used to determine crude fat based on the Soxhlet Extraction Method of AOAC, (1990). The fat obtained was cooled in a desiccator, weighed and expressed as a percentage of the initial weight of the sample using the formula:

$$\% \text{ Crude Fat} = \frac{\text{Weight of fat residue}}{\text{Weight of MOLM sample}} \times 100$$

2.3. Crude Fibre Content

Triplicate 2 g of defatted samples (from crude fat determination) were used to determine crude fibre using the method of the Association of Official Analytical Chemists (AOAC, 1990). Crude fibre content and was expressed as a percentage of the initial weight of the sample using the formula.

$$\% \text{ Crude Fibre} = \frac{\text{Crude fibre content}}{\text{Weight of Fresh Sample}} \times 100$$

2.4. Crude Protein

The method described by Association of Official Analytical Chemists (AOAC, 1990) and using an Automatic Kjeldahl Protein Analyser (BIOBASE®, China) was used to determine total nitrogen amount in triplicate samples. Two grams (2 g) of MOLM was used to determine percentage total nitrogen in samples. A nitrogen-to-protein conversion factor of 6.25 was used to estimate protein in MOLM samples. % Crude Protein = % Nitrogen x 6.25

2.5. Ash Content

A 10.0 g MOLM samples were placed in drying oven at 105 °C for 24 hours. After cooling, triplicate 2.0 g samples were put in a muffle furnace (Ceramic Fibre Muffle Furnace, China) and the temperature increased gradually to 550 ± 5 °C for at least 8 hours until constant weight was obtained. Ash content was determined as follows:

$$\text{Ash \%} = \frac{\text{Weight of Ash}}{\text{Weight of MOLM Sample}} \times 100$$

2.6. Carbohydrate (Nitrogen-Free Extract/NFE)

Total carbohydrate content (%) was calculated by difference method using the formula:

$$\text{NFE \%} = 100 - (\text{Moisture} + \text{Crude Protein} + \text{Crude Fibre} + \text{Crude Lipid} + \text{Crude Ash}).$$

2.7. Mineral Analysis

The remaining inorganic material was cooled, weighed and further used for the determination of mineral contents. An ash solution was prepared by dissolving the ash in 100 ml of 1 M HCl. Three samples (5 g each) were heated at 550 °C and shaken overnight to ensure fair distribution of heat in the mixture. Concentrated nitric acid was used to dissolve the ash, filtered and diluted to 50 ml with deionized water. An Atomic Absorption Spectrometer (ZEEnit P Series- Analytik Jena AG, Germany) with air/acetylene flame (for Fe, Mg, Mn, K and Zn) and N₂O/acetylene flame (for Ca and P) and graphite furnace and the absorbance of the samples read at wavelengths of 422.7 nm (Ca), 248.3 nm (Fe), 766.5 nm (K), 285.2 nm (Mg), 279.5 nm (Mn), 214.9 nm (P) and 213.9 nm (Zn). The results for mineral contents were expressed as mg/kg DW.

2.8. Energy Estimation of MOLM

The bomb calorimeter method (Parr 6400 Calorimeter, USA) was used to estimate energy of triplicate samples of MOLM1 and MOLM2. For each leaf meal, 1.0 g was put in the jacket of the bomb calorimeter at a temperature of 30 °C for 10 mins and at the end of the analysis results were printed out as the energy values for MOLM samples.

2.9. Phytochemical Assay of *Moringa Oleifera* Leaf Meal (MOLM)

2.9.1. Qualitative Screening

To prevent the destruction of active compounds, MOLM samples were kept away from direct sun light and high temperatures. Serial extractions using 200 g moringa leaf powder in 500 ml 70 % ethanol in Erlenmeyer flask were done. At two hourly intervals the mixture was shaken during day-time for 3 days. Using Whatman's No.1 Filter Paper in Buchner funnel with a suction pump the mixture was decanted and filtered. The filtrate was concentrated *in vacuo* using a rotary evaporator. Colorimetric methods described by Gupta *et al.*, (2014) were used to detect alkaloids, steroids, terpenoids, tannins, saponins and coumarins in MOLM.

2.10. Test for Alkaloids

Six (6) drops of 2 % hydrochloric acid was used to dissolve 1 mg of dried extract. After dividing the solution into three, 2 ml of distilled water was added to one portion to serve as reference. The formation of a precipitate after adding 2 drops of Dragendorff's reagent and potassium iodide confirmed the presence of alkaloids. The formation of yellowish white precipitate after adding 2 drops of Mayer's reagent indicated the presence of alkaloids.

2.11. Test for Steroids and Triterpenoids

To 1 mg of dried extracts, 0.5 ml of acetic anhydride was added to dissolve it followed by 0.5 ml of chloroform. To the pipetted solution in a dry test tube 1 ml of concentrated sulphuric acid was added to the bottom of the tube. The occurrence at the interface between the two liquids of a brown-red ring and a green supernatant showed the presence of steroids and triterpenoids.

2.12. Test for Tannins

Three (3) drops of dilute ferric chloride was added to 1 mg of plant extracts dissolved in 1.5 ml of distilled water. Blackish blue color and green blackish colour indicated the presence of gallic tannins and catechol tannins respectively.

2.13. Test for Saponins

To 1 mg of plant extract, 3 drops of dimethyl sulfoxide and 5 ml of distilled water were added and shaken. Foam which lasted more than 15 min was positive for saponins.

2.14. Test for Coumarins

To one portion of 1 mg of extract dissolved in 2 ml of distilled water, 0.5 ml of 10 % ammonia solution was added. The other portion was used as reference. Coumarins and its derivatives showed as intense fluorescence under ultraviolet light.

2.15. Phytochemical Assay of *Moringa Oleifera* Leaf Meal (MOLM)

2.15.1. Quantitative Analysis of Phytochemicals

Quantitative phytochemical analysis of MOLM was determined by methods described below:

2.16. Quantification of Alkaloids

The method by Harbone (1998), was used to quantify alkaloids in MOLM. In this method, a 200 ml of 20 % acetic acid in ethanol was added to 5 g sample of the MOLM and dissolved in a 250 ml beaker and allowed to stand for a period of 4 hours. The mixture was then filtered and the extracts was concentrated to about one quarter of its original volume using a water-bath. Few drops of concentrated ammonium hydroxide were added to the extract to

complete the precipitation process. The precipitate was collected using Whatman Filter paper no. 42 and then weighed.

2.17. Quantification of Tannins

The method by Van-Burden and Robinson (1981) was used to determine tannin content in MOLM. After weighing 500 mg of the sample into a 100 ml plastic bottle, 50 ml of distilled water was added and shaken in a mechanical shaker for a period of one hour. The solution was then filtered into a 50 ml volumetric flask and filled up to the graduated mark. A pipette was then used to transfer 5 ml of the filtrate into a test tube and mixed with 3 ml of 0.1M FeCl₃ in 0.1N HCl and 0.008 M potassium ferrocyanide. Within 10 minutes, a spectrophotometer was used to measure the absorbance at 120 nm wavelength. A standard sample using tannin acid to get 100 ppm and a blank sample with colour developed were prepared and read at the same wavelength.

2.18. Quantification of Saponins

The content of saponins in MOLM was determined using the methods described by Obadoni and Ochuko (2001). A 20 g sample of ground MOLM was dispersed in a 100 ml of 20 % ethanol and the suspension stirred continuously for a period of 4 hours over a hot water bath at a temperature of 55 °C. After filtering the mixture, the residue was re-extracted using another 200 ml of 20 % ethanol. The extract was heated on a water bath at about 90 °C till the volume reduced to 40 ml and the concentrate transferred into a 250 ml separator funnel, 20 ml of diethyl ether added and shaken vigorously. The ether layer was discarded while the aqueous layer was recovered. The process of purification was repeated and 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated over a water bath to evaporate it and dried to constant weight in an oven.

2.19. Quantification of Total Phenolics

The phenolics content in MOLM was determined using the method outlined by Santhi and Sengottuvel (2016). 5 ml extract of fat free sample boiled with 50 ml of ether for 15 minutes was pipetted into a 50 ml flask and 10 ml of distilled water, and 2 ml of ammonium hydroxide solution added. To 5 ml of the extract in a 50 ml flask, 10 ml of distilled water, 2 ml of ammonium hydroxide solution and 5 ml of concentration amyl alcohol were added and left for 30 minutes to react for colour change. A spectrophotometer was used to read the absorbance of the solution at 505 nm wavelength.

2.20. Quantification of Total Flavonoids

The aluminum chloride colorimetric method described by Miliauskas, *et al.* (2004) using catechin as a standard was used to quantify the total flavonoid content of MOLM. 1 ml of the test sample and 4 ml of water were added and put into a 10 ml volumetric flask. 0.3 ml of 5 % sodium nitrite, 0.3 ml of 10 % aluminum chloride was added after 5 minutes. Again, 2 ml of 1 M sodium hydroxide was added to the reaction mixture after 6 minutes of incubation at room temperature. The final volume was increased with distilled water until it reached 10 ml and the absorbance measured spectrophotometrically at 510 nm wavelength against a blank sample and expressed as catechin equivalents (CE) per gram dry weight sample (mg CE/g DW).

2.21. Quantification of Oxalates

The method by Ndubuaku *et al.*, (2015) was used to quantify oxalate in the MOLM. With this method, a 20 ml of 30 % HCl was added to 2 g of leaf sample placed in a 300 ml flask and allowed to stand for a period of 20 minutes. To this solution 40 g of ammonium sulphate was added and allowed to stand for 30 minutes, filtered into a 250 ml volumetric flask and diluted to the graduated mark using 30 % HCl. To 10 ml of the filtrate in 100 ml centrifuge tube, 30 ml of diethyl ether was added. Concentrated NH₄OH solution was added to adjust the pH of the solution such that the colour changed from salmon pink to faint yellow. The mixture was then centrifuged at 10,000 g for a period of 15 minutes and then decanted into a 250 ml conical flask. Finally, the decant was titrated with 0.1 M potassium tetraoxomanganate (VII) (KMnO₄).

The volume was recorded and % Oxalate calculated as follows:

$$\% \text{ Oxalate} = \frac{(\text{Titre} \times \text{mol KMnO}_4 \times \text{DF (12.5)}) \times 100}{\text{Weight of sample}}$$

2.22. Determination of Phytate Content

The method by Ndubuaku *et al.*, (2015) was used to determine the phytate content in MOLM. 0.5 g of moringa leaves was weighed into a 500 ml flat bottom flask. At room temperature, the sample was placed in a shaker and extracted with 100 ml of 2.4 % HCl for 1 hour, after which the mixture was decanted and filtered. Distilled water was used to dilute 5 ml of the pipetted filtrate to the 25 ml mark. For 10 ml of diluted sample, 15 ml of sodium chloride was added and the solution passed through an ampetraisen, and 15 ml of 0.7 M sodium chloride added. Absorbance of the solution was read at 520 nm wavelength. A blank was prepared and absorbance read at the same wavelength and a standard curve produced and from this the concentration of phytate in the test sample was determined or quantified.

2.23. Antioxidant Assay of MOLM

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method (Brand-Williams *et al.*, 1995; Dudonne *et al.*, 2009) was used for antioxidant assay of MOLM. MOLM extract concentrations of 0.02 mg/ml, 0.04 mg/ml, 0.06 mg/ml, 0.08 mg/ml and 0.1 mg/ml were prepared with methanol and 5 ml of each was mixed with 0.5 ml of 1 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol. In triplicate, test tubes were incubated for 30 min at room temperature and the absorbance measured at 517 nm with a Perkin Elmer Spectrum Two FTIR Spectrometer (PerkinElmer Inc., USA). Ascorbic acid of same concentrations was used as standard and test solutions. The difference in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as % Scavenging of DPPH Radical. Scavenge ability of the DPPH radical was calculated as follows:

$$\text{Scavenging Effect (\%)} = \left(1 - \frac{A_s}{A_c}\right) \times 100$$

A_s = the absorbance of the sample at $t = 0$ min.

A_c = the absorbance of the control at $t=30$ min.

IC 50 = concentrations of test samples (MOLM and ascorbic acid) to reduce the initial DPPH concentration by 50 %.

IC 50 values were computed from the % scavenging obtained by plotting logarithmic curves.

2.24. Statistical Analysis

Experimental data was analyzed using Analytical Software STATISTIX 9.0. (2008) and the means separated by least significant difference (LSD) at 5 %.

3. Results

3.1. Proximate Composition and Energy of *Moringa Oleifera* Leaf Meals

The results for proximate analysis of *Moringa oleifera* leaf meals are presented in Table 1. No significant differences ($P>0.05$) occurred in proximate compositions.

3.2. Mineral Composition of *Moringa Oleifera* Leaf Meal

Minerals detected in MOLMs were Ca, Fe, K, Mg, Mn, P and Zn. Mineral concentrations of leaf meals are presented in Table 2. Appreciable amounts of Ca, Fe, K, Mg, Mn, P and Zn were recorded in MOLM1 and MOLM2. No significant differences occurred in all the minerals for MOLM1 and MOLM2 ($P>0.05$).

3.3. Phytochemical Composition of *Moringa Oleifera* Leaf Meal

Qualitative and quantitative phytochemical assays of MOLM are presented as follows:

3.4. Qualitative Assay of MOLM

Phytochemical screening was positive for alkaloids, coumarins, flavonoids, saponins, steroids, sterols, tannins and terpenoids. Phytochemicals that were qualitatively detected in MOLM are shown in Table 3.

3.5. Quantitative Phytochemical Assay of MOLM

Quantities of phytochemicals present in MOLM have been presented in Table 4. No significant differences occurred in the quantities of phytochemicals in MOLM1 and MOLM2 ($P>0.05$).

3.6. Antioxidant Property of *Moringa Oleifera* Leaf Meal

The capability of MOLM and ascorbic acid to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is presented in Table 5. In this study, IC₅₀ (concentration of test samples to reduce DPPH concentration by 50 %) values for MOLM1, MOLM2 and ascorbic acid were 7.17 µg/ml, 7.13 µg/ml and 27.91 µg/ml, respectively.

4. Discussion

4.1. Proximate Composition of *Moringa Oleifera* Leaf Meal

Moisture recorded in this study for *Moringa oleifera* leaf meals are in agreement with Witt, (2013) but higher than values reported by Aye and Adegun, (2013), Valdez-Solana *et al.*, (2015), Sultana, (2020) and Peñalver *et al.*, (2022). Variations in moisture content of MOLM in this study and other authors could be attributed to differences in maturity of leaves and the degree of shade drying. Younger leaves tend to be much more succulent and contain more moisture than older leaves and so require longer periods of shade-drying to attain a required level of dryness. Leaves that are dried under warm and well ventilated environments lose moisture quicker than those dried under cold and poorly ventilated conditions or environments with low wind circulation or wind flow (Ramsumair *et al.*, 2014). Consequently, varying levels of drying may have affected dry matter content.

Crude protein values of 27.42 and 27.72 % were higher than values recorded by Valdez-Solana *et al.*, (2015), but in the range of 22.23 to 30.3 % (Witt, 2013; Aye and Adegun, 2013; Sultana, 2020; Peñalver, *et al.*, 2022). According to Ayissiwe *et al.*, (2011), crude protein levels of multipurpose tree leaves are greater than those of grasses and vegetable shrubs and often exceed 20 %. Crude protein content of MOLM in this study is significant and

valuable because it could be a suitable partial substitute for relatively expensive plant protein sources such as soya bean meal and wheat bran in agreement with [Su and Chen, \(2020\)](#).

Crude fat in this study were lower than values reported earlier ([Aye and Adegun, 2013](#); [Valdez-Solana, et al., 2015](#); [Peñalver et al., 2022](#)) but within the range reported by [Sultana \(2020\)](#). The mean crude fat content recorded in the leaf meals in this study constituted a valuable source of lipids especially essential fatty acids. Their inclusion in diets will lead to substantial contributions to the fatty acid profile of formulated feeds.

Crude fibre of MOLM1 and MOLM2 were comparable with values reported earlier ([Witt, 2013](#); [Valdez-Solana et al., 2015](#); [Aye and Adegun, 2013](#)) but higher than other reports ([Aye and Adegun 2013](#); [Sultana, 2020](#)). However, crude fibre recorded by [Peñalver et al., \(2022\)](#) was exceedingly higher and approximately thrice more than values recorded in this study. Differences in fibre content of MOLM can be attributed to the level of maturity of leaves and proportion of twigs and fibrous veins that are milled with leaves to produce leaf meal. Older leaves tend to be more fibrous than younger leaves because of the higher levels of cellulose, lignin and pentosans silica and other mineral matter content. The extent of sieving and diameter of mesh (2.0–3.0 mm) for sieving milled leaves could also influence the level of roughage and the proportion of fibre in moringa leaf meals.

Crude ash values in this study were higher than figures recorded by [Aye and Adegun \(2013\)](#), [Sultana, \(2020\)](#) and [Peñalver et al., \(2022\)](#) but in close range with some documented values ([Witt, 2013](#); [Valdez-Solana et al., 2015](#)). The high ash content of 12.98 % and 13.39 % in this study indicated that a high inorganic residue was obtained after burning off the organic matter of the leaf meal. The inorganic residue which is an indicator of mineral content implied that MOLM is rich in mineral elements. Variations in ash content and those recorded by other authors ([Witt, 2013](#); [Valdez-Solana et al., 2015](#)) could be due to differences in edaphic factors, stage of plant growth, maturity of leaves and analytical techniques.

Carbohydrate levels were higher than data from [Peñalver et al., \(2022\)](#) but in accord with values reported by [Aye and Adegun \(2013\)](#). Levels of carbohydrate in this study were also found to be lower than values reported by [Valdez-Solana et al., \(2015\)](#) and [Sultana, \(2020\)](#). Carbohydrate values of 34.21 and 34.74 % in this study indicated a high level of soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in the leaf meals. Variations of carbohydrate levels in this study and those recorded from aforementioned studies could be due to the innate or inherent composition of leaf meals and procedures for analysis. Carbohydrates represent the available energy in the leaf meal and therefore contribute significantly to the total energy in the leaf meal. Gross energy of MOLM in this study was higher than 14.79 MJ/kg reported by [Moyo et al., \(2011\)](#) but in agreement with value reported earlier ([El-Ramady et al., 2014](#)), indicating high levels of energy in moringa leaf meals in this study.

4.2. Mineral Composition of *Moringa Oleifera* Leaf Meal

Differences in elemental composition and concentration of minerals (Ca, Fe, K, Mg, Mn, P and Zn) in MOLM in this study ([Table 2](#)) and moringa leaf meals by other authors ([Moyo et al., 2011](#); [Aye and Adegun 2013](#); [Witt, 2013](#); [Valdez-Solana et al., 2015](#); [Sultana, 2020](#)) and especially [Peñalver et al., 2022](#) who recorded exceptionally higher values may be due to variations in the availability of macro- and micro-nutrients in different geographical locations and soils. Variations in concentration of mineral elements recorded in moringa leaf meals in this study and other studies in Limpopo Province of South Africa by [Moyo et al., \(2011\)](#), Nigeria by [Aye and Adegun \(2013\)](#), Lombardia and San Pedro in Mexico by [Valdez-Solana et al., \(2015\)](#) and other locations around the world is largely due to the differences in the chemistry and composition of soils. These variations can make nutrients unavailable in certain soils, or in forms that cannot be utilized by plants ([Morgan and Connolly, 2013](#); [Stein et al., 2017](#)). Other factors such as rainfall pattern and intensity (availability of soil water), soil acidity, soil texture and compaction all affect nutrient availability and hence their uptake by plants ([Stein et al., 2017](#); [Javed et al., 2022](#)).

4.3. Phytochemical Composition of *Moringa Oleifera* Leaf Meal

Phytochemicals that were qualitatively detected in MOLM is in accord with reports by [Nweze and Nwafor \(2014\)](#) and [Abd Rani et al., \(2018\)](#). Quantities of alkaloids in this study were in close range with values reported by [Ajayi et al., \(2017\)](#) and [Nweze and Nwafor \(2014\)](#). Flavonoids quantified in MOLM in this study (0.28 mg/g or 28 mg/100g) was lower than values (5.06 – 12.16 mg/g) from earlier studies by some authors ([Leone et al., 2015](#)), but higher than 3.56 ± 0.03 g/100g reported by [Nweze and Nwafor, \(2014\)](#). Quantities of saponins were in close agreement with [Nweze and Nwafor \(2014\)](#). Tannins were higher than values reported by ([Leone et al., 2015](#)), but similar to values by [Ajayi et al., \(2017\)](#). Phenols in this study were higher than quantities reported by [Ademiluyi et al., \(2018\)](#). Although oxalates were within the range reported in literature, phytate levels were lower than values reported by ([Leone et al., 2015](#)).

Differences in quantities of phytochemicals in MOLM in this study and other authors in literature ([Leone et al., 2015](#)) could be attributed to several factors. Different agroclimatic conditions have been reported as possible causes of differences in quantities of phytochemicals, total phenolic compounds and antioxidant potential of *Aloe vera* ([Kumar et al., 2017](#)). By extension, this could be a cause of the variation of the quantities of phytochemicals of MOLM in this study and other moringa leaf meals analysed from different locations around the world. Different methods and intensity of drying (freeze-drying, sun, air and oven drying) *Moringa oleifera* leaves have influence on phytoconstituents (phenolics, flavonoids, vitamin C, tannin, saponin, phytate, oxalate, alkaloid, cardenolides, and cardiac glycosides) and antioxidant capacities ([Ademiluyi et al., 2018](#)).

Phytochemicals and bioactive agents in MOLM are responsible for the pharmaceutical, biochemical, industrial properties and intermediary nutritional roles. It is popular due to its nutritional and medicinal properties. Alkaloids,

coumarins, flavonoids, saponins, steroids, sterols, tannins and terpenoids authenticate the usefulness of moringa leaves in ethnomedicine (Nweze and Nwafor 2014). Spontaneously hypertensive rats fed ethanolic extracts at a dose of 1000 mg/kg each for 14 days significantly reduced systolic and diastolic blood pressure in rats and hence showed hypotensive effects (Kumolosasi *et al.*, 2021). Anti-hypertensive properties of the leaf meal are attributed to alkaloids. This property according to Qiu *et al.*, (2014) is due to their role in the dilation of blood vessels and hence their use as remedy for sympathomimetic conditions (Kamyab *et al.*, 2021). The antipyretic property in animals and anti-malarial effect in humans is due to alkaloids. Although they possess local anesthetic properties, they are seldom used for this purpose (Cushnie *et al.*, 2014).

Alkaloids and saponins are hypercholesterolemic in nature. The hypocholesterolemic ability of MOLM is enhanced by saponins and particularly sterols which cause reduction of LDL blood cholesterol without affecting HDL cholesterol. The anti-inflammatory role of MOLM has been attributed to flavonoids. Flavonoids have been found to prevent oxidation of LDL cholesterol and as such are useful in the cure of heart disease and arteriosclerosis (Salvamani *et al.*, 2014). Blood thinning and anticoagulant features of coumarin and tannins cause reduction in blood pressure and so are effective in reducing cardiovascular diseases (Romagnolo and Selmin, 2012).

Anti-tumour/anti-cancer properties of MOLM are due to alkaloids (Ahmad *et al.*, 2017; Uzor, 2020), saponins (Yacoub, 2015; Marrelli *et al.*, 2016; Ballout *et al.*, 2019; Mondal *et al.*, 2019), flavonoids (Panche *et al.*, 2016; Adamczak *et al.*, 2020; Kopustinskiene *et al.*, 2020), coumarin (Mandlik *et al.*, 2016; Sharifi-Rad *et al.*, 2021), tannins and their associated bioactive compounds (Youness *et al.*, 2021). Another benefit of MOLM is its antimicrobial properties attributed to flavonoids and polyphenols (Royani *et al.*, 2023) while antifungal and antiprotozoal properties are due to coumarin and alkaloids (Qiu *et al.*, 2014; Sharifi-Rad *et al.*, 2021; Tshabalala *et al.*, 2020). Alkaloids and terpenoids have anti-cough properties and so reduce or prevent irritation in throats of animals (Tholl, 2015; Gopalakrishnanb, 2016; Negi *et al.*, 2020).

The ability of MOLM in boosting reproductive performance is associated with the production of hormones such as estrogen, cortisol, testosterone and estradiol (Gunaherath *et al.*, 2014). Treatment of urinary tract infections in nephropathic patients (Romani, 2021), urinary tract infection (Noce *et al.*, 2021), diarrhea, dysentery (Hoste *et al.*, 2022).

Phytic acid and oxalate have been reported to interfere with the intake, digestion, absorption, utilization of nutrients, and in some instances cause adverse effects in animals. They may also be considered as non-nutritive compounds with positive health effects by consumers (Popova and Mihaylova, 2019).

4.4. Antioxidant Property of *Moringa Oleifera* Leaf Meal

In this study, IC₅₀ of MOLM1 and MOLM2 extracts (7.13 and 7.13 µg/ml respectively) were much lower than that of vitamin C or standard drug (27.91 µg/ml). This meant that the concentrations of test samples (MOLM and ascorbic acid) to reduce the initial DPPH concentration by 50 % were 7.17 µg/ml, 7.13 µg/ml and 27.91 µg/ml for MOLM and ascorbic acid, respectively. This indicated that MOLM extracts had better antioxidant abilities when compared with ascorbic acid because of the high DPPH scavenging potential observed in MOLM than ascorbic acid (Vitamin C).

MOLM had scavenging ability four times that of ascorbic acid. The results of this experiment are in consonance with that of Ndhala *et al.*, (2014) who reported 2 – 5 times scavenging abilities of dried leaves from different cultivars of moringa. The high antioxidant property of *Moringa oleifera* leaves according to Ndhala *et al.*, (2014) can be attributed to bioactive compounds such as 4-[4'-O-acetyl- α -L-rhamnosyloxy) benzyl] isothiocyanate, niazirin, niazirinin, niaziminin A and B. Antioxidant properties of MOLM are due to saponins, tannins and terpenoids (Ndhala *et al.*, 2014). Flavonoids also have strong antioxidant properties (Panche *et al.*, 2016). Terpenoids in particular are strong antioxidants of carotenoids oxidation and lipid peroxidation and found to be important in human health (Negi *et al.*, 2020). Immunity of animals is boosted and modulated by saponins and tannins respectively (Ndhala *et al.*, 2014).

The antioxidant property of MOLM makes it appropriate as an ingredient to reduce rancidity in feed and impede free radical formation and rancidity in meat.

Conclusions

Proximate composition of MOLM in this study showed adequate quantities of crude protein, carbohydrate, minerals and energy, and therefore serve as a suitable ingredient that can partially replace soya bean meal and wheat bran (conventional plant protein sources). The higher scavenging ability by MOLM of DPPH radical makes it a suitable natural antioxidant in the prevention of rancidity in feed and, consequently, reduction or inhibition of lipid oxidation in live birds and meat. The detection of alkaloids, coumarins, flavonoids, saponins, steroids, sterols, tannins and terpenoids, and quantities of alkaloids, flavonoids, saponins, tannins, total phenols, oxalates and phytates indicated that MOLM in this study had useful nutritional and medicinal phytochemicals with properties which could promote growth and health of farm animals.

Authors' Contributions

William K. J. Kwenin conceived the idea, carried out data collection, analysis, interpretation of data, drafting and submission of the manuscript for publication.

Competing Interests

There is no competing interest.

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Table-1. Proximate Composition and Energy of *Moringa oleifera* Leaf Meal

Proximate Fraction	Composition (%)		LSD	Sig
	MOLM1	MOLM2		
Moisture	9.77	9.50	0.51	ns
Crude Protein	27.42	27.72	1.35	ns
Crude Fat	4.55	4.65	0.65	ns
Crude Fibre	10.56	10.37	1.15	ns
Ash	13.39	12.98	0.82	ns
Carbohydrate	34.21	34.74	2.05	ns
GE (MJ/kg)	17.77	17.82	0.98	ns

LSD= Least Significant Difference; Sig= Significance; ns= (P>0.05); GE= Gross Energy.

Table-2. Mineral Composition of *Moringa oleifera* Leaf Meals

Minerals	MOLM1 (mg/kg)	MOLM2 (mg/kg)	LSD	Sig
Calcium (Ca)	655.93	656.31	19.04	ns
Iron (Fe)	70.21	71.60	3.81	ns
Potassium (K)	257.72	266.58	8.91	ns
Magnesium (Mg)	73.49	69.739	2.13	ns
Manganese (Mn)	12.32	12.64	1.29	ns
Phosphorus (P)	586.31	597.69	21.17	ns
Zinc (Zn)	16.75	16.68	0.90	ns

LSD= Least Significant Difference; Sig= Significance; ns= (P>0.05).

Table-3. Phytochemicals in *Moringa oleifera* Leaf Meal

Phytochemical Screening	MOLM1	MOLM2
Alkaloids	+	+
Coumarins	+	+
Flavonoids	+	+
Saponins	+	+
Sterols	+	+
Steroids	+	+
Tannins	+	+
Terpenoids	+	+

+= Presence of phytochemical

Table-4. Quantities of Phytochemicals in *Moringa oleifera* leaf meal

PARAMETER	MOLM 1	MOLM 2	LSD	Sig
Alkaloids	2.66 %	2.59 %	0.17	ns
Flavonoids	28 mgCE/g	27 mgCE/g	6.12	ns
Saponins	1.54 %	1.57 %	0.09	ns
Tannins	49.26 mgTAE/g	49.76 mgTAE/g	1.64	ns
Total Phenols	106.44 mg/g	106.42 mg/g	3.73	ns
Oxalate	1.42 mg/g	1.10 mg/g	0.87	ns
Phytic Acid	11.72 mg/g	11.51 mg/g	1.06	ns

LSD= Least significant difference; Sig= Significance; ns= (P>0.05)

Table-5. Ability of *Moringa oleifera* Leaf Meal and Ascorbic Acid (Vit. C) to Scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

Sample	IC50 (µg/ml)
MOLM1	7.17
MOLM2	7.13
Ascorbic acid	27.91

IC 50= Concentration of test samples to reduce DPPH concentration by 50%.